

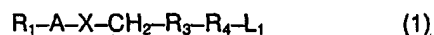
WO 2005/085470

PCT/EP2005/050900

- 64 -

### Claims

1. A compound of formula (1)



wherein

A is a group recognized by O<sup>6</sup>-alkylguanine-DNA alkyltransferases (AGT) as a substrate;

X is oxygen or sulfur;

R<sub>1</sub> is a group -R<sub>2</sub>-L<sub>2</sub> or a group R<sub>5</sub>;

R<sub>2</sub> and R<sub>4</sub> are, independently of each other, a linker;

R<sub>3</sub> is an aromatic or a heteroaromatic group, or an optionally substituted unsaturated alkyl, cycloalkyl or heterocyclyl group with the double bond connected to CH<sub>2</sub>;

R<sub>5</sub> is arylmethyl or heteroarylmethyl or an optionally substituted cycloalkyl, cycloalkenyl or heterocyclyl group;

L<sub>1</sub> is a label, a plurality of same or different labels, a bond connecting R<sub>4</sub> to A forming a cyclic substrate, or a further group -R<sub>3</sub>-CH<sub>2</sub>-X-A-R<sub>1</sub>; and

L<sub>2</sub> is a label or a plurality of same or different labels.

2. The compound according to claim 1 of formula (1) wherein

A is a heteroaromatic group containing 1 to 5 nitrogen atoms;

X is oxygen;

R<sub>1</sub> is a group -R<sub>2</sub>-L<sub>2</sub> or a group R<sub>5</sub>;

R<sub>2</sub> and R<sub>4</sub> are, independently of each other, a straight or branched chain alkylene group with 1 to 300 carbon atoms, wherein optionally

(a) one or more carbon atoms are replaced by oxygen, in particular wherein every third carbon atom is replaced by oxygen, e.g. a polyethyleneoxy group with 1 to 100 ethyleneoxy units;

(b) one or more carbon atoms are replaced by nitrogen carrying a hydrogen atom, and the adjacent carbon atoms are substituted by oxo, representing an amide function -NH-CO-;

(c) one or more carbon atoms are replaced by oxygen, and the adjacent carbon atoms are substituted by oxo, representing an ester function -O-CO-;

WO 2005/085470

PCT/EP2005/050900

- 65 -

- (d) the bond between two adjacent carbon atoms is a double or a triple bond, representing a function  $-\text{CH}=\text{CH}-$  or  $-\text{C}\equiv\text{C}-$ ;
- (e) one or more carbon atoms are replaced by a phenylene, a saturated or unsaturated cycloalkylene, a saturated or unsaturated bicycloalkylene, a bridging heteroaromatic or a bridging saturated or unsaturated heterocyclyl group;
- (f) two adjacent carbon atoms are replaced by a disulfide linkage  $-\text{S}-\text{S}-$ ; or a combination of two or more, especially two or three, alkylene and/or modified alkylene groups as defined under (a) to (f) hereinbefore, optionally containing substituents;

$\text{R}_3$  is phenyl, an unsubstituted or substituted mono- or bicyclic heteroaryl group of 5 or 6 rings atoms comprising zero, one, two, three or four ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, with the proviso that at least one ring carbon atom is replaced by a nitrogen, oxygen or sulfur atom, 1-alkenyl, 1-alkinyl, 1-cyclohexenyl with 3 to 7 carbon atoms, or an optionally substituted unsaturated heterocyclyl group with 3 to 12 atoms and 1 to 5 heteroatoms selected from nitrogen, oxygen and sulfur, and a double bond in the position connecting the heterocyclyl group to methylene  $\text{CH}_2$ ;

$\text{R}_5$  is optionally substituted phenylmethyl or naphthylmethyl; optionally substituted heteroarylmethyl wherein heteroaryl is a mono- or bicyclic heteroaryl group comprising zero, one, two, three or four ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, with the proviso that at least one ring carbon atom is replaced by a nitrogen, oxygen or sulfur atom, and which has 5 to 12 ring atoms; optionally substituted cycloalkyl with 3 to 7 carbon atoms; optionally substituted cycloalkenyl with 5 to 7 carbon atoms; optionally substituted saturated or unsaturated heterocyclyl with 3 to 12 atoms, and 1 to 5 heteroatoms selected from nitrogen, oxygen and sulfur;

$\text{L}_1$  is one or a plurality of same or different labels selected from a spectroscopic probe, a magnetic probe, a contrast reagent, a molecule which is one part of a specific binding pair which is capable of specifically binding to a partner, a molecule that is suspected to interact with other biomolecules, a library of molecules that are suspected to interact with other biomolecules, a molecule which is capable of crosslinking to other molecules, a molecule which is capable of generating hydroxyl radicals upon exposure to  $\text{H}_2\text{O}_2$  and

WO 2005/085470

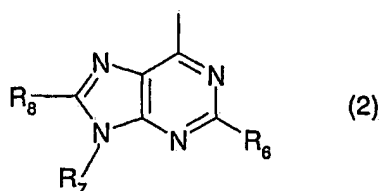
PCT/EP2005/050900

- 66 -

ascorbate, a molecule which is capable of generating reactive radicals upon irradiation with light, a molecule covalently attached to a solid support, a nucleic acid or a derivative thereof capable of undergoing base-pairing with its complementary strand, a lipid or other hydrophobic molecule with membrane-inserting properties, a biomolecule with desirable enzymatic, chemical or physical properties, a bond connecting  $R_4$  to A forming a cyclic substrate, and a further group  $-R_3-CH_2-X-A-R_1$ ; and

$L_2$  is one or a plurality of same or different labels selected from a spectroscopic probe, a magnetic probe, a contrast reagent, a molecule which is one part of a specific binding pair which is capable of specifically binding to a partner, a molecule that is suspected to interact with other biomolecules, a library of molecules that are suspected to interact with other biomolecules, a molecule which is capable of crosslinking to other molecules, a molecule which is capable of generating hydroxyl radicals upon exposure to  $H_2O_2$  and ascorbate, a molecule which is capable of generating reactive radicals upon irradiation with light, a molecule covalently attached to a solid support, a lipid or other hydrophobic molecule with membrane-inserting properties, and a biomolecule with desirable enzymatic, chemical or physical properties.

3. The compound according to claim 1 of formula (1) wherein the group  $R_1-A$  is a purine radical of formula (2)



wherein  $R_6$  is hydrogen, hydroxy or unsubstituted or substituted amino; and one of  $R_7$  and  $R_8$  is  $R_1$  and the other one is hydrogen.

4. The compound according to claim 3 of formula (1) wherein X is oxygen and  $R_3$  is phenyl.

WO 2005/085470

PCT/EP2005/050900

- 67 -

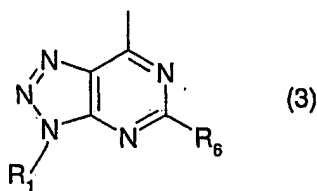
5. The compound according to claim 3 of formula (1) wherein X is oxygen and R<sub>3</sub> is thienyl.
6. The compound according to claim 3 of formula (1) wherein the group R<sub>1</sub>-A is a purine radical of formula (2), R<sub>6</sub> is unsubstituted amino, R<sub>7</sub> is R<sub>1</sub>, R<sub>8</sub> is hydrogen, and X is oxygen.
7. The compound according to claim 3 of formula (1) wherein the group R<sub>1</sub>-A is a purine radical of formula (2), R<sub>6</sub> is unsubstituted amino, R<sub>7</sub> is a group -R<sub>2</sub>-L<sub>2</sub>, R<sub>8</sub> is hydrogen, and X is oxygen.
8. The compound according to claim 7 wherein L<sub>2</sub> is a spectroscopic probe.
9. The compound according to claim 7 wherein L<sub>1</sub> and L<sub>2</sub> are spectroscopic probes.
10. The compound according to claim 9 wherein L<sub>1</sub> and L<sub>2</sub> represent a fluorescence donor / fluorescence quencher pair.
11. The compound according to claim 10 wherein L<sub>1</sub> and L<sub>2</sub> represent a FRET pair.
12. The compound according to claim 3 of formula (1) wherein the group R<sub>1</sub>-A is a purine radical of formula (2), R<sub>6</sub> is unsubstituted amino, R<sub>7</sub> is a group R<sub>5</sub>, R<sub>8</sub> is hydrogen, and X is oxygen.
13. The compound according to claim 12 wherein R<sub>5</sub> is cyclopentyl.
14. The compound according to claim 3 of formula (1) wherein the group R<sub>1</sub>-A is a purine radical of formula (2), R<sub>6</sub> is unsubstituted amino, R<sub>7</sub> is hydrogen, R<sub>8</sub> is R<sub>1</sub>, and X is oxygen.
15. The compound according to claim 3 of formula (1) wherein the group R<sub>1</sub>-A is a purine radical of formula (2), R<sub>6</sub> is unsubstituted amino, R<sub>7</sub> is hydrogen, R<sub>8</sub> is a group -R<sub>2</sub>-L<sub>2</sub>, and X is oxygen.

WO 2005/085470

PCT/EP2005/050900

- 68 -

16. The compound according to claim 15 wherein  $L_2$  is a spectroscopic probe.
17. The compound according to claim 15 wherein  $L_1$  and  $L_2$  are spectroscopic probes.
18. The compound according to claim 17 wherein  $L_1$  and  $L_2$  represent a fluorescence donor / fluorescence quencher pair.
19. The compound according to claim 18 wherein  $L_1$  and  $L_2$  represent a FRET pair.
20. The compound according to claim 15 wherein  $L_2$  is a molecule representing one part of a specific binding pair.
21. The compound according to claim 15 wherein  $L_2$  is a molecule covalently attached to a solid support.
22. The compound according to claim 15 wherein  $L_2$  is a cell membrane transport enhancer group.
23. The compound according to claim 1 of formula (1) wherein the group  $R_1-A$  is an 8-azapurine radical of formula (3)



wherein the substituent  $R_6$  is hydrogen, hydroxy or unsubstituted or substituted amino.

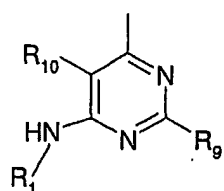
24. The compound according to claim 23 of formula (1) wherein X is oxygen and  $R_3$  is phenyl.
25. The compound according to claim 23 of formula (1) wherein the group  $R_1-A$  is an 8-azapurine radical of formula (3),  $R_6$  is unsubstituted amino,  $R_1$  is a group  $-R_2-L_2$ , and X is oxygen.

WO 2005/085470

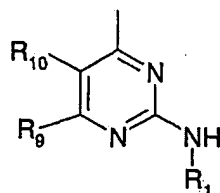
PCT/EP2005/050900

- 69 -

26. The compound according to claim 25 wherein  $L_2$  is a spectroscopic probe.
27. The compound according to claim 25 wherein  $L_1$  and  $L_2$  are spectroscopic probes.
28. The compound according to claim 27 wherein  $L_1$  and  $L_2$  represent a fluorescence donor / fluorescence quencher pair.
29. The compound according to claim 28 wherein  $L_1$  and  $L_2$  represent a FRET pair.
30. The compound according to claim 1 of formula (1) wherein the group  $R_1$ -A is a pyrimidine radical of formula (4a) or (4b)



(4a)



(4b)

wherein  $R_9$  is hydrogen, halogen, lower alkyl with 1 to 4 carbon atom or amino, and  $R_{10}$  is hydrogen, halogen, lower alkyl with 1 to 4 carbon atoms, amino, nitro or nitroso.

31. The compound according to claim 30 of formula (1) wherein X is oxygen and  $R_3$  is phenyl.
32. The compound according to claim 30 of formula (1) wherein the group  $R_1$ -A is a pyrimidine radical of formula (4a) or (4b),  $R_1$  is a group  $-R_2-L_2$ , and X is oxygen.
33. The compound according to claim 32 wherein  $L_2$  is a spectroscopic probe.
34. The compound according to claim 32 wherein  $L_1$  and  $L_2$  are spectroscopic probes.
35. The compound according to claim 34 wherein  $L_1$  and  $L_2$  represent a fluorescence donor / fluorescence quencher pair.

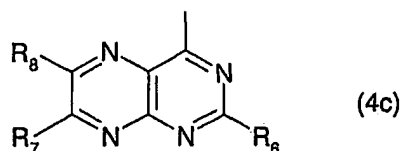
WO 2005/085470

PCT/EP2005/050900

- 70 -

36. The compound according to claim 35 wherein  $L_1$  and  $L_2$  represent a FRET pair.

37. The compound according to claim 1 of formula (1) wherein the group  $R_1-A$  is a pteridine radical of formula (4c)



wherein  $R_6$  is unsubstituted or substituted amino; and one of  $R_7$  and  $R_8$  is  $R_1$  and the other one is hydrogen.

38. The compound according to claim 37 of formula (1) wherein  $X$  is oxygen and  $R_3$  is phenyl.

39. The compound according to claim 37 of formula (1) wherein the group  $R_1-A$  is a pteridine radical of formula (4c),  $R_6$  is unsubstituted amino,  $R_7$  is hydrogen,  $R_8$  is  $R_1$ ,  $R_1$  is a group  $-R_2-L_2$ , and  $X$  is oxygen.

40. The compound according to claim 39 wherein  $L_2$  is a spectroscopic probe.

41. The compound according to claim 39 wherein  $L_1$  and  $L_2$  are spectroscopic probes.

42. The compound according to claim 41 wherein  $L_1$  and  $L_2$  represent a fluorescence donor / fluorescence quencher pair.

43. The compound according to claim 42 wherein  $L_1$  and  $L_2$  represent a FRET pair.

44. A method for detecting and/or manipulating a protein of interest, wherein the protein of interest is incorporated into an AGT fusion protein, the AGT fusion protein is contacted with a compound of formula (1) according to any one of claims 1 to 38, and

WO 2005/085470

PCT/EP2005/050900

- 71 -

the AGT fusion protein is detected and optionally further manipulated using the label  $L_1$  in a system designed for recognising and/or handling the label.

45. The method according to claim 44, wherein in the compound of formula (1) label  $L_2$  is a solid support, and the AGT fusion protein contacted with the compound of formula (1) is separated from the compound of formula (1) by filtration or centrifugation or separation of magnetic beads.

46. The method according to claim 44, wherein in the compound of formula (1) label  $L_1$  is one member and label  $L_2$  the other member of two interacting spectroscopic probes  $L_1 / L_2$ , and the AGT fusion protein is detected by fluorescence.

47. The method according to claim 44 for detecting and/or manipulating a protein of interest, wherein the protein of interest is fused with a mutant AGT, the mutant AGT fusion protein is contacted with a mixture of

- (a) a compound of formula (1) wherein  $R_1$  is a group  $R_5$  and which is not recognized by the mutant AGT, and
- (b) another compound of formula (1) recognized by the mutant AGT fusion protein,

and the mutant AGT fusion protein is detected and optionally further manipulated using the label in a system designed for recognizing and/or handling the label.